Isozyme Data and the Taxonomy of Checkerspot Butterflies (*Euphydryas*)

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Abstract. The taxonomy of butterflies in the genus *Euphydryas* was investigated using allozyme frequency data from 19 presumptive loci. Six *Euphydryas* species and three species in the tribe Melitaeini (*Chlosyne acastus*, *Chlosyne palla*, and *Melitaea phoebe*) were included in the study. This set of species included at least one representative each of Higgins' four proposed genera within *Euphydryas*. Dendrograms derived using UPGMA, neighbor-joining, distance Wagner, and maximum likelihood clustering methods were used to establish the similarity of the sampled taxa. The analyses do not support Higgins' generic rearrangement.

Key Words: Allozymes, electrophoresis, phenogram

Introduction

Checkerspot butterflies of the genus *Euphydryas* have become a key system for testing theories in population biology in the field. An equivalent body of information on population dynamics, ecology, and genetics such as is available for checkerspots probably does not exist for a suite of populations in any other group of animals. Work with the *Euphydryas* system and comparative work with other butterflies has led to the development of generalities which may prove valid for at least the majority of herbivorous insects. These generalities include the importance of gene flow in evolution, the "regulation" of population size, the frequency of extinction, and selection versus neutrality in accounting for allozyme variability (Ehrlich et al. 1975, Ehrlich et al. 1980, Ehrlich and White 1980, Meuller et al. 1985, Weiss et al. 1987, Brussard et al. 1989, and Baughman et al. 1990).

Some of the same features that make *Euphydryas* butterflies so attractive for hypothesis testing in population biology, however, have made the genus a difficult one for systematists. Several of the species are highly polytypic and show complex variation in size, wing patterns and coloration, and a variety of ecological characteristics. This has resulted in a proliferation of trinomials; for example, Miller and Brown (1981) list 61 subspecies among nearctic species and Ferris (1989) lists 62 (but also see Scott 1986).

In spite of the common occurrence of extensive intraspecific variation, nevertheless, diagnostic characters in wing pattern and genitalia exist which allow separation of most forms at the species level. Traditionally, 14 species

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have been recognized, six of which are Nearctic, and eight Palearctic (Higgins 1950, Ehrlich and Ehrlich 1961, Howe 1975, Miller and Brown 1981). However, three of the Nearctic species, *Euphydryas anicia*, *E. chalcedona*, and *E. colon*, appear to represent a classic "ring of races" with reproductive isolation between populations on the terminal ends rather than separate monophyletic lineages (Scott 1986, Brussard et al. 1989). Thus, *Euphydryas anicia* and *E. colon* are properly treated as synonyms of *E. chalcedona*, the priority name, reducing the number of Nearctic species to four.

Higgins (1978) divided *Euphydryas* into four genera, *Hypodryas*, consisting of four palearctic and one Nearctic species; *Eurodryas*, consisting of four Palearctic species; *Occidryas*, consisting of four Nearctic species (three of which were synonomized, making two); and *Euphydryas*, consisting of a single Nearctic species. The currently recognized species of *Euphydryas* (*sensu latu*) are shown in Table 1, along with Higgins' (1978) proposed generic rearrangement.

The splitting of checkerspots into four genera was strongly criticized by Ehrlich and Murphy (1982) for a number of reasons, and their criticisms were reinforced by genetic information presented by Brussard et al. (1985). After Brussard et al. (1985) was completed, we obtained statistically reasonable samples of *Euphydryas desfontainii* and *E. aurinia*, two representatives of Higgins' proposed genus *Eurodryas*. This additional material now allows us to determine levels of genetic differentiation in six of the 12 species of *Euphydryas s.l.*, at least one of which is in each of Higgins' putative genera.

The first question we address is whether the genetic differentiation observed among these species is more suggestive of that typically seen at the intrageneric or the intergeneric level among other butterfly groups. The second question concerns the relationships of these species to each other through the use of gene frequency data with various clustering methods. More specifically, does the arrangement of species in Table 1 seem to represent a natural grouping of species of *Euphydryas s.l.* in light of their geographic distributions and other characteristics?

Methods

A total of 18 populations were sampled for this study (Table 2). Higgins' "Occidryas" group is represented by 228 individuals from four populations of *Euphydryas editha* and 493 individuals from seven populations of *E. chalcedona*. These populations were taken from a much larger array sampled for other studies (e.g. Brussard et al. 1989, Vawter and Wright 1986, and unpublished data) and were selected to maximize geographical representation in these species. All other samples were collected in the summer of 1986. Higgins' "Euphydryas" group is represented by 58 individuals from two populations of *E. phaeton*, "Hypodryas" by a sample of 20 individuals from one population of *E. gillettii*, and "Eurodryas" by 92 individuals from two populations of *E. desfontainii* and 27 individuals from one population of *E. aurinia*. We used two species of Nearctic *Chlosyne* and one species of Palearctic *Melitaea* for both comparison and as outgroups in the analyses. Both of these genera are included with *Euphydryas* in the tribe Melitaeini. These samples included 28 individuals from one population of *Melitaea phoebe*, 20 individuals from one population of *Chlosyne acastus*, and 28 individuals from one population of *C. palla*.

Table 1. Higgins' four genera proposed for the genus *Euphydryas*.

Higgins (1978)	"Hypodryas"	"Eurodryas"	"Occidryas"	"Euphydryas"
	Euphydryas maturna	*Euphydryas aurinia*[1]	*Euphydryas chalcedona*[1]	*Euphydryas phaeton*[1]
	E. intermedia	*E. desfontainii*[1]	*E. editha*[1]	
	E. iduna	*E. alexandrina*		
	E. cynthia	*E. orientalis*		
	E. gillettii[1]			

[1] Species that were included in the present study.

Individual butterflies were assayed for variability at 19 presumptive loci which could be reliably scored in side-by-side comparisons of all six *Euphydryas* species and three outgroup species run on the same gels. These loci are AAT-1,2, AGP (G3P), DIA, GAPD, GDH, GPI, HBDH, HK-1, IDH-1, LDH, MDH-1,2, MPI, PEP-GL, PGD, PGM, SOD-1,2 (isozyme names and buffer systems used are listed in Brussard et al. [1985] with the exceptions that HK-1 resolved on buffer C, and AGP [G3P] resolved on buffer 4). Loci are numbered and electromorphs were designated in order of increasing anodal mobility.

Table 2. Sample locations and sizes used in the allozyme analyses of *Euphydryas* taxonomy.

Taxon	Sample Location	Sample Size
Euphydryas editha	Gunnison Co., CO, USA	59
	Thurston Co., WA, USA	73
	Riverside Co., CA, USA	57
	Mariposa Co., CA, USA	39
E. chalcidona	Gunnison Co., CO, USA	79
	Nye Co, NV, USA	49
	Maricopa Co, AZ, USA	56
	Stanislaus Co., CA, USA	59
	Santa Clara Co., CA, USA	58
	Inyo Co, CA, USA	41
	Jackson Co., OR, USA	78
	Polk Co., OR, USA	73
E. phaeton	Franklin Co., MO, USA	28
	Otsega Co., NY, USA	30
E. gillettii	Teton Co., WY, USA	20
E. desfontainii	Campo Real, Spain	40
	Rhonda, Spain	52
E. aurinia	El Escorial, Spain	27
Melitaea phoebe	Campo Real, Spain	28
Chlosyne acastus	Nye Co., NV, USA	20
C. palla	Gunnison Co., CO, USA	28

All gel runs included an *Euphydryas editha* standard inserted into every tenth slot in the gels, and each electromorph was identified by its mobility relative to that of the most common electromorph in *E. editha* at each locus. Phenotypes were recorded directly from the gels. Electromorph frequencies were determined by direct count from the observed phenotypes. Population-level data from each species represented by more than one sample were combined to represent electromorph frequencies for each species as a whole.

Nei's (1978) unbiased and Roger's genetic distances, UPGMA phenograms, and rooted Wagner trees were estimated using BIOSYS-1 (Swofford and Selander 1981). A neighbor-joining (Saitou and Nei 1987) dendrogram was generated using the genetic distances obtained from BIOSYS-1. A maximum likelihood network was generated directly from the observed electromorph frequencies using the CONTML subroutine in PHYLIP 3.5 (Felsenstein 1993).

Results

The number of electromorphs per locus ranged from three to 10 over all the operational taxonomic units (OTU's) used in this study with all loci being polymorphic in at least one species. The frequency of each electromorph present in each species as a whole is shown in Table 3. As can be seen in Table 3, most electromorphs at a given locus are shared by several species, although often at quite different frequencies. Only five electromorphs proved to be diagnostic (i.e. fixed or nearly fixed in one taxon while not present in the others). DIAd, LDHi, and SOD-1g appear to be diagnostic of *Chlosyne*, GDHd of *Euphydryas aurinia*, and LDHb of *E. gillettii*. However, because some sample sizes are relatively small, some of these electromorphs really may not be unique to these taxa. Several other electromorphs such as G3Pa, DIAa, GDHc, HBDHb, HBDHg, and SOD-1a are fixed or nearly fixed in one or more species, but segregate as rare variants in others (Table 3).

Composite Phenogram

The topologies of phenograms derived using UPGMA based on Nei's unbiased distances and Roger's distances (Table 4), neighbor-joining based on Nei's unbiased distances (Table 4), the distance Wagner method, and maximum likelihood were all very similar. A "composite" phenogram that shows the common pattern of clustering in all the phenograms derived from these methods is shown in Figure 1. The composite phenogram is scaleless, i.e. it shows the pattern of genetic similarity derived by all the methods used without presenting genetic distances among OTU's.

The most salient point to be drawn from the composite phenogram (Figure 1) is that the North American *Euphydryas* species form a discrete cluster. The next most similar set of species to these is the European *Euphydryas* which constitute Higgins' (1978) "Eurodryas."

Discussion

The similarity among the tree topologies derived by a number of procedures using the *Euphydryas* data suggests that the general pattern of genetic similarity among these taxa has been captured by the analyses. Kim et al. (1993) tested this assumption by estimating the accuracy of trees derived by maximum parsimony, UPGMA, and neighbor-joining using simulated data. The results of the analysis by Kim et al. (1993) suggested that the assumed correlation between concordance of topologies from a number of tree-estimating algorithms and tree accuracy, as made in a number of studies (e.g. Dowling and Brown 1989, Zink and Avise 1990, Giannasi et al. 1992, and Valdebenito et al. 1992), is probably correct. This result lends confidence in interpreting the results of the present study.

Brussard et al. (1985) used a smaller number of taxa in a similar analysis to the present one to argue against splitting of the genus *Euphydryas* into four genera as suggested by Higgins (1978). The inclusion of additional Old World *Euphydryas* samples and outgroups in the present analysis strengthens

Table 3. Allele frequencies at polymorphic loci for six *Euphydryas* species and three additional species in the tribe Melitaeini. Sample sizes are provided.

Species[1]		Ee	Ec	Ep	Eg[2]	Ed	Ea	Mp	Ca	Cp[2]
Sample size		228	493	58	20	92	27	28	20	28
No. populations		4	7	2	1	2	1	1	1	1
Locus (E.C. no.)	Allele									
AAT-1										
(2.6.1.1)	A	0.011	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000
	B	0.031	0.020	0.009	0.000	0.967	0.981	0.000	0.000	0.000
	C	0.941	0.960	0.957	1.000	0.000	0.019	0.000	0.000	0.000
	E	0.020	0.020	0.026	0.000	0.000	0.000	1.000	0.150	0.875
	G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.850	0.054
	H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.054
AAT-2										
(2.6.1.1)	B	0.000	0.000	0.009	0.000	1.000	1.000	0.036	0.000	0.000
	C	0.851	0.990	0.991	1.000	0.000	0.000	0.964	0.800	0.446
	E	0.149	0.010	0.000	0.000	0.000	0.000	0.000	0.200	0.429
	F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125
DIA-1										
(1.8.1.4)	A	0.011	0.010	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	B	0.989	0.990	0.000	0.000	1.000	1.000	0.000	0.000	0.000
	D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
	E	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
GAPDH										
(1.2.1.12)	A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.196
	B	0.000	0.000	0.000	0.000	0.082	0.000	0.179	1.000	0.804
	C	1.000	1.000	1.000	1.000	0.918	0.833	0.821	0.000	0.000
	E	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000
GDH-1										
(1.4.1.2)	B	0.969	0.970	1.000	1.000	1.000	0.000	1.000	1.000	0.000
	C	0.031	0.030	0.000	0.000	0.000	0.000	0.000	0.000	1.000
	D	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000
GPI-1										
(5.3.1.9)	A	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.059	0.000	0.112	0.100	0.000	0.000	0.000	0.000	0.000
	C	0.570	0.040	0.836	0.900	0.038	0.000	0.000	0.000	0.000
	E	0.101	0.350	0.017	0.000	0.940	0.944	0.018	0.325	0.464
	F	0.070	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
	G	0.149	0.550	0.009	0.000	0.022	0.037	0.143	0.675	0.536
	H	0.039	0.050	0.000	0.000	0.000	0.019	0.446	0.000	0.000
	I	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	J	0.000	0.000	0.000	0.000	0.000	0.000	0.179	0.000	0.000
	K	0.000	0.000	0.000	0.000	0.000	0.000	0.214	0.000	0.000
G3P-1										
(1.1.1.8)	A	0.011	0.000	0.000	0.025	0.000	0.000	1.000	0.000	0.000
	B	0.989	1.000	1.000	0.975	1.000	1.000	0.000	1.000	1.000

HBDH1										
(1.1.1.30)	A	0.000	0.020	0.000	0.000	0.022	0.000	0.000	0.000	0.000
	B	0.039	0.040	0.009	1.000	0.978	0.056	0.000	0.000	0.000
	C	0.000	0.000	0.000	0.000	0.000	0.000	0.821	0.000	0.000
	D	0.851	0.640	0.991	0.000	0.000	0.000	0.000	0.000	0.000
	E	0.000	0.000	0.000	0.000	0.000	0.944	0.000	0.000	0.000
	F	0.110	0.300	0.000	0.000	0.000	0.000	0.179	0.000	0.000
	G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
HK-01										
(2.7.1.1)	B	0.020	0.020	0.983	0.000	0.489	0.389	0.000	0.000	0.000
	C	0.691	0.690	0.017	0.025	0.511	0.611	0.000	0.000	0.000
	D	0.289	0.290	0.000	0.975	0.000	0.000	1.000	1.000	1.000
IDH-1										
(1.1.1.42)	B	0.000	0.090	0.009	0.000	0.000	0.000	0.000	0.000	0.000
	D	0.989	0.740	0.991	1.000	1.000	0.593	0.018	0.000	0.000
	F	0.011	0.130	0.000	0.000	0.000	0.370	0.982	0.925	0.536
	G	0.000	0.020	0.000	0.000	0.000	0.037	0.000	0.075	0.464
	H	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-1										
(1.1.1.27)	A	0.000	0.000	0.000	0.000	1.000	1.000	0.893	0.900	0.000
	B	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
	E	0.000	0.060	0.922	0.000	0.000	0.000	0.107	0.100	0.000
	F	1.000	0.080	0.078	0.000	0.000	0.000	0.000	0.000	0.000
	G	0.000	0.810	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	H	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
MDH-1										
(1.1.1.37)	B	0.000	0.000	0.000	0.000	0.109	0.000	0.000	0.000	0.000
	C	0.011	0.040	0.000	0.050	0.022	0.000	0.089	1.000	1.000
	E	0.941	0.950	0.957	0.950	0.739	1.000	0.821	0.000	0.000
	F	0.050	0.010	0.043	0.000	0.130	0.000	0.071	0.000	0.000
	G	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000
MDH-2										
(1.1.1.37)	B	0.000	0.000	0.741	0.000	0.000	0.000	0.000	0.000	0.000
	C	0.961	1.000	0.259	1.000	1.000	1.000	1.000	1.000	1.000
	D	0.039	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MPI-1										
(5.3.1.8)	B	0.039	0.090	0.000	0.000	0.022	0.000	0.000	0.000	0.000
	C	0.910	0.610	0.043	1.000	0.929	0.093	0.000	0.000	0.054
	E	0.039	0.170	0.638	0.000	0.049	0.093	0.411	0.125	0.946
	F	0.011	0.110	0.224	0.000	0.000	0.463	0.411	0.000	0.000
	G	0.000	0.020	0.103	0.000	0.000	0.185	0.179	0.875	0.000
	J	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000
PEP-1										
(3.4.13.11)	B	0.020	0.010	0.000	0.000	0.000	0.000	0.821	1.000	1.000
	C	0.969	0.710	0.931	1.000	0.989	1.000	0.179	0.000	0.000
	D	0.011	0.230	0.069	0.000	0.011	0.000	0.000	0.000	0.000
	E	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000

PGD-1									
(1.1.1.44)	B	0.000	0.020	0.009	1.000	0.000	0.000	0.000	1.000	0.946
	C	1.000	0.970	0.991	0.000	0.000	1.000	0.982	0.000	0.054
	D	0.000	0.010	0.000	0.000	0.891	0.000	0.018	0.000	0.000
	E	0.000	0.000	0.000	0.000	0.060	0.000	0.000	0.000	0.000
	F	0.000	0.000	0.000	0.000	0.049	0.000	0.000	0.000	0.000
PGM-1										
(5.4.2.2)	A	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.000	0.000	0.000	0.000	0.098	0.000	0.000	0.000	0.000
	C	0.000	0.080	0.000	0.000	0.011	0.074	0.036	0.000	0.000
	D	0.070	0.210	0.000	0.000	0.891	0.778	0.964	0.000	0.000
	E	0.750	0.290	0.043	0.000	0.000	0.148	0.000	0.000	0.000
	F	0.171	0.280	0.569	0.050	0.000	0.000	0.000	0.000	0.000
	G	0.000	0.120	0.379	0.950	0.000	0.000	0.000	0.000	0.000
	I	0.000	0.020	0.009	0.000	0.000	0.000	0.000	0.500	1.000
	J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000
	K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.400	0.000
SOD-1										
(1.15.1.1)	A	0.000	0.000	0.000	0.000	0.011	0.000	1.000	0.000	0.000
	B	0.000	0.010	0.000	0.000	0.011	0.000	0.000	0.000	0.000
	D	1.000	0.990	0.974	1.000	0.978	1.000	0.000	0.000	0.000
	E	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000
	G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
SOD-2										
(1.15.1.1)	A	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000
	B	1.000	1.000	1.000	1.000	1.000	1.000	0.982	1.000	1.000

[1] Ee-*Euphydryas editha*, Ec-*Euphydryas chalcedona*, Ep-*Euphydryas phaeton*, Eg-*Euphydryas gillettii*, Ed-*Euphydryas desfontainii*, Ea-*Euphydryas aurinia*, Mp-*Melitaea phoebe*, Ca-*Chlosyne acastus*, and Cp-*Chlosyne palla*.
[2] Data previously reported in Brussard et al. (1985).

Table 4. Nei's (1978) unbiased genetic distances above the diagonal and Roger's modified genetic distances (Wright 1978) below diagonal for six species of *Euphydryas* and three comparitive taxa also in the tribe Melitaeini.

	1	2	3	4	5	6	7	8	9
1. *E. editha*	——	0.091	0.290	0.345	0.426	0.481	0.837	1.000	1.000
2. *E. chalcedona*	0.268	——	0.301	0.367	0.405	0.426	0.705	0.995	1.000
3. *E. phaeton*	0.468	0.464	——	0.364	0.699	0.704	0.926	1.000	1.000
4. *E. gillettii*	0.591	0.522	0.533	——	0.526	0.838	0.936	0.954	1.000
5. *E. desfontainii*	0.553	0.529	0.669	0.620	——	0.234	0.946	1.000	1.000
6. *E. aurinia*	0.575	0.534	0.664	0.723	0.430	——	0.838	1.000	1.000
7. *M. phoebe*	0.696	0.640	0.722	0.745	0.731	0.697	——	0.714	0.936
8. *C. acastus*	0.778	0.723	0.809	0.757	0.783	0.791	0.666	——	0.249
9. *C. palla*	0.810	0.760	0.835	0.800	0.835	0.818	0.724	0.443	——

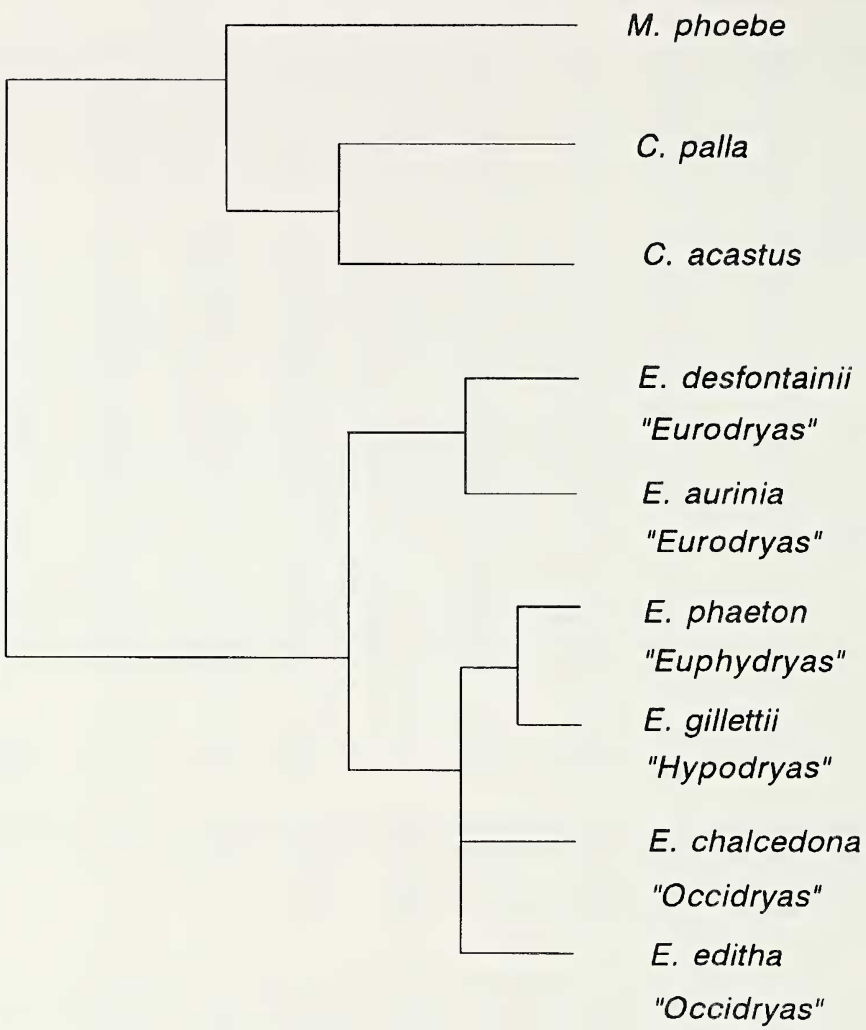

Figure 1. A composite phenogram based on UPGMA, neighbor-joining, Wagner, and maximum likelihood analyses of allozyme frequencies from 19 loci for six species of *Euphydryas* and three comparative species also in the tribe Melitaeini. Higgins' (1978) putative genera are in quotation marks. The composite phenogram does not support the generic rearrangement of *Euphydryas sensu latu* as proposed by Higgins.

this conclusion. Estimates of genetic distances among *Euphydryas* species are consistent with the present nomenclatural arrangement for the genus (Brussard et al. 1985). The taxa from *Euphydryas s.l.* form a cluster separate from the comparative taxa, *Chlosyne acastus*, *C. palla*, and *Melitaea phoebe*, in all topologies. This also strongly suggests that little justification exists for splitting *Euphydryas s.l.* into four genera. Furthermore, the level of differentiation between the comparative taxa and the *Euphydryas* spp. on the UPGMA

phenograms indicates that *Euphydryas s. l.* is a valid taxonomic entity (Brussard 1985). Finally, these data suggest that *Chlosyne acastus* and *C. palla* are genetically very similar. Nei's (1978) unbiased genetic distance between these two species (0.249) is among the lowest in Table 2 and is of a magnitude suggestive of semispecies or sibling species for other insect taxa (Brussard et al. 1985).

Higgins "Eurodryas" (*E. aurinia* and *E. desfontainii*) clusters within the *Euphydryas* branch on the composite dendrogram, suggesting that those species are measurably differentiated from the other *Euphydryas* taxa. Eurodryas may serve as an appropriate nomen to refer to these taxa as a European "species group" or subgenus. That interpretation should be viewed as tentative, however, because two obstensible species of "Eurodryas," *Euphydryas alexandrina* and *E. orientalis*, were not included in the present analysis.

We conclude that the allozyme data presented above do not justify splitting the genus *Euphydryas* into four genera as suggested by Higgins (1978). The consistent clustering of *Euphydryas* spp. as a pool of species distinct from closely related members of the tribe Melitaeini using a number of different phenetic clustering methods demonstrates the substantive cohesion of *Euphydryas sensu latu.*

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